TI13
+Y12
1921

YALE UNIVERSITY LIBRARY
3 9002 06712 4538

THE TRANSPLANTATION OF EMBRYONIC OVARIES AND TESTES OF THE GUINEA PIG WITH REFERENCE TO THEIR COTRANSPLANTABILITY

Paul Gonick

1955

MUDD
LIBRARY
Medical

YALE

MEDICAL LIBRARY

Digitized by the Internet Archive
in 2017 with funding from
Arcadia Fund

https://archive.org/details/transplantationo00goni

The Transplantation of Embryonic Ovaries and Testes of the Guinea Pig with Reference to their Cotransplantability

Paul Gonick, B A

New York University, 1951

James Hudson Brown Fellow
Summer 1953

Presented to the Faculty of the

School of Medicine, Yale University,

in Candidacy for the Degree of

Doctor of Medicine

Department of Pathology

1955

YALE MEDICAL
AUG 1955
LIBRARY

ACKNOWLEDGEMENTS

I would like to thank Dr. Harry S.N. Greene for his guidance and advice of this project. In addition I would like to express my appreciation to Dr. Harold S.F. Fleming, Elizabeth K. Harvey, and Richard G. Sutphin for their interest and aid.

This thesis was aided by a grant from the James Hudson Brown Research Fund.

CONTENTS

	Page
Introduction	1
Methods and Materials	4
Donor tissue	
Transplantation into the anterior chamber	
Transplants to the testicle	
Ovariectomy	
Castration	
Harvesting of tissues	
Results	8
Ovary and testis cotransplants in normal animals.	
Ovary and testis cotransplants in castrate animals	
Cotransplants of ovaries and testes in pregnant animals	
Bilateral gonadal transplants	
Normal hosts with testis transplants	
Castrate hosts with testis transplants	
Castrate hosts with ovary transplants	
Embryonic ovary transplanted to adult testes	
Second generation transplants into the anterior chamber of the eye	
Miscellaneous ovary-testes cotransplants	
The role of embryo age in transplantability	
Duration of transplants	
Microscopic description of the transplants	
Summary of results	
Discussion	19
Transplant factors	
Host factors	
Technique factors	
Transplants in pregnant hosts	
Embryonic age and transplantability	
Cotransplantability	
Maturation of the transplants	
Summary	27
Photomicrographs	28
References	37

CONTENTS

Page

Introduction 1

Methods and Materials 4

Donor tissue
Transplantation into the anterior chamber
Transplants to the testicle
Ovariectomy
Castration
Harvesting of tissues

Results 8

Ovary and testis cotransplants in normal animals.
Ovary and testis cotransplants in castrate animals
Cotransplants of ovaries and testes in pregnant animals
Bilateral gonadal transplants
Normal hosts with testis transplants
Castrate hosts with testis transplants
Castrate hosts with ovary transplants
Embryonic ovary transplanted to adult testes
Second generation transplants into the anterior chamber
of the eye
Miscellaneous ovary-testes cotransplants
The role of embryo eye in transplantability
Duration of transplants
Microscopic description of the transplants
Summary of results

Discussion 19

Transplant factors
Host factors
Technique factors
Transplants in pregnant hosts
Embryonic age and transplantability
Cotransplantability
Maturation of the transplants

Summary 27

Photomicrographs 28

References 37

INTRODUCTION

The pathophysiological causes of cancer have been and still are a puzzling and yet fascinating field of investigation. Many of the theories expounded have involved embryonic tissues as the source of cancerous growth. If autonomy and anaplasia are accepted as the chief characteristics of cancer, (33) it can readily be seen why embryonic tissues have been used in cancer research. As early as 1884, attempts have been made to produce sarcomas, teratomas and other cancerous tissue from embryonic transplants (61). Greene has produced teratomatous tissue by transplanting rabbit embryos to the testes of guinea pigs. Occasionally transplants of these tissues showed "changes highly suggestive of carcinoma or sarcoma" (22). He has also noted modifications in the differentiation of embryonic tissue, notably in the lung, where squamous metaplasia of the bronchial epithelium and epithelialization of alveoli (so-called lung adenomas) are most common (23).

Willis (61) summarizes the attempts to produce teratomas and other tumors from embryonic transplants (up to 1934) as follows:

> Embryos or parts of embryos, implanted whole or minced or pulped into adult animals of like species, may suffer any of three fates: (i) the tissue may be rapidly and completely absorbed, or (ii) they may survive, grow, and then suffer resorbtion, or (iii) they may grow to considerable masses of mixed differentiated tissues which may persist for long periods, even for the lifetime of the host animal...The masses obtained from embryo tissues are not teratomas; they are merely differentiating grafts.

Moreover, Greene (22) casts doubts on tumors reported in the literature which arise from untreated transplants, while Rous (2,3) states that no matter how stimulated experimentally, embryonic tissues have formed nothing more than self limited teratoid growths. However, he

admits that "a neoplasm has occasionally arisen, but the very rare carcinomas have occurred under equivocal conditions, while the sarcomas might well have derived from the stroma supplied to the implant from the host." (55). He further reports the formation of malignant epidermal tumors from the painting of methylcholanthrene on embryonic skin transplants. Earlier and later investigators (2,3,24, 34,45,53) have shown that with the use of methylcholanthrene or Fowlers solution, carcinomas and sarcomas can readily be produced from embryonic tissues. Loeb (40) speculates on the production of cancer by such adjuvants as methylcholanthrene and Fowlers solution as being caused by an excessive growth stimulus given to susceptible tissues which then autocatalytically continue to grow. Although these chemicals are not normally found in the body, there are substances in most body fluids that are cancerogenic. The sex hormones are, according to Loeb, (41)

> the only cancerogenic substance known so far which are normal constituents of the animal body and which here exert a normal function mainly and primarily as growth stimulators in the accessory sex organs, and as a result of this function, may cause the development of cancer in the organs in which they normally induce growth processes.

Administration of estrogens has resulted in interstitial cell tumors of the testis in certain strains of mice (4,5,18,31). Granulosa cell tumors and luteomas were produced by excess production of pituitary gonadotrophins by the ingenious method of intrasplenic ovarian transplants in castrate male and female mice (36).

The transplantation of embryonic ovary and testes in close association was planned for the study of: (i) the possible tumor inducing properties of such transplants and (ii) the transplantability of embryonic

gonadal tissues (8,10,12,13,14,17,19,30,35,37,39,47,48,51,54,57,58,59,60).

METHODS AND MATERIALS

Mixed breed guinea pigs, three to four months old, were used in all the eye transplants. Although the anterior chamber was somewhat smaller in these animals than in fully grown adults, it has been noted by Rous (50) that growing animals are better hosts for embryonic transplants.

Donor Tissues

The embryonic ovaries and testes used in the transplants were obtained under aseptic conditions by cesarean section after the mother had been sacrificed in a chloroform jar. The embryos were placed in sterile Petri dishes and the gonadal tissues removed. These were placed in a separate Petri dish with either normal saline, amniotic fluid or embryonic skin added to protect the small pieces of gonads from dehydration. Embryos of 4.5 to 11 cm. in size were used. They were approximately forty-five to sixty-five days of gestation (15,30).

Bookhout (6) has shown that it is possible to differentiate ovary from testes on the twenty-seventh day of gestation and the medulla and cortex by the forty-fifth day. In the embryos used, it was usually possibly, by gross morphology, to differentiate testes from ovaries.

The testicular tissue was cut into two or more pieces. If the ovaries were over one millimeter in diameter, they, too, were split before being transplanted. In all but four animals, only litter mate gonads were used in the cotransplants. In so far as possible, it was also attempted to obtain controls from the same litter used in the experimental group.

Transplantation into the Anterior Chamber of the Eye

Loeb (43) has noted that transplants are influenced both in the

number of "takes" and growth by the site of transplantation. The anterior chamber of the eye is the most advantageous site known for endocrine organ transplants (23,41). In addition the technique is simple; a high number of transplants "take"; growth can be followed by gross observation; they can readily be separated from the host tissue; and there is less inflammatory reaction than in other sites.

Greene's (20) transplantation technique was used in all the eye transplants.* In most animals, this was done in the right eye only.

After fastening the animal to a board by all four extremities, cocaine (5%) was instilled into the eye. While the eye was being anesthesized, the tissue (ovary, testis, or ovary and testis) was either placed in the trocar (#19) with fine forceps or aspirated into the trocar by withdrawing the stylet. A corneal knife was then used to make an incision at the superior corneoscleral junction. The trocar was inserted into the anterior chamber and the tissue expelled. The tissue was then pushed into the iridocorneal junction. This was accomplished by external pressure on the cornea applied with the aid of a blunt instrument. No sutures were necessary. In order to avoid infections, strict sterile technique must be used. Antibiotics may also be of use.

Transplants to the Testicle

Many investigators have used the testicle as a transplantation site for gonadal tissues (22,38,39,51,58,59). Although not as good as the anterior chamber, successful transplants have been reported.

The testicles of adult guinea pigs were cleaned with 70% ethanol. An embryonic ovary was inserted into a lumbar puncture needle (#19). The testis was visualized by a nick in the scrotum. The lumbar needle was

*For a beautiful photographic sequence of the technique, see Patino's (44) work.

then introduced into the middle of the testis and the ovary was expelled by the stylet. The lumbar needle was then examined to make certain that the tissue had been left in the testis. No sutures were placed in the scrotum. Some animals had transplants introduced bilaterally.

Ovariectomy

A bilateral lumbar subcostal approach was used for ovariectomy. The back of the animal was shaved from T5 to L5. The skin was cleaned with 70% alcohol and a one cm. incision made just below the costo-vertebral angle. The incision was deepened down to the peritoneum, keeping as bloodless a field as possible. The proximity of the ovary was located by using the peritoneum as a glass bottomed boat. When a small white tubular structure was visualized, the peritoneum was pierced with forceps and the tubular structure grasped and brought out into the wound. The ovary was also pulled into the wound by this procedure. It was then ligated and removed. The muscles and skin were closed with silk in separate layers. The procedure was then repeated on the other side.

Castration

The scrotum was shaved and cleaned with 70% ethanol. Using an iris scissor, a small 3/4 cm. incision was made in the ventro-lateral area of each scrotal sac. The incision was carried through the tunica vaginalis. This allowed the scrotal contents, when messaged from the abdomen towards the incision, to be extruded from the scrotum. The testis and epididymis were then clamped, ligated, and removed. The spermatic cord and vessel were then replaced into the abdomen via the inguinal canal with small forceps. The procedure was repeated on the other side.

The edges of the scrotum were approximated. No sutures were necessary.

Intramuscular nembutal anesthesia was used in the above procedures.

All spayed and castrated animals were allowed to recover from the operative procedure for two or three weeks before receiving transplants.

Harvesting of Tissues

In the eye transplants, the hosts were sacrificed with liberal doses of chloroform. The eye was then removed and the transplant with its attachments dissected free, placed on blotting paper and fixed in 10% formalin. Representative sections of all successful transplants were taken and stained with Harris' hematoxylin and eosin.

The testicular sites of transplantation were dissected free from the dead animal and fixed in formalin. Thin sections were then cut with a razor blade and examined. The site of the transplant was then used for microscopic sections.

Table 1

Normal animals with ovary and testis cotransplants*

Embryos	Host: Male #	Male %	Female #	Female %	Total #	Total %
Total	7	100	6	100	13	100
Male takes	2	29	3	50	5	39
Female takes	0	0	0	0	0	0
Absorbed	0		1	17	1	8
Regressed	5	71	2	33	7	53

*Only non-pregnant cotransplants from the same litter and under 14 weeks duration in the eye.

Table 2

Castrate animals with ovary and testis cotransplants.

Embryos	Host: Male #	Male %	Female #	Female %	Total #	Total %
Total	5	100	7	100	12	100
Male takes	3	60	4	57	7	58
Female takes	1	20	0	0	1	83
Male and female takes	0	0	0	0	0	0
Absorbed	0		1	14	1	83
Regressed	1	20	2	29	3	25

RESULTS

Ovary and Testis Cotransplants in Normal Animals

Thirteen normal animals (Table 1) had cotransplants of ovary and testes tissue taken from the same litter. Of these, about half (7) were male and half (6) female. The embryos used varied from seven to ten cm. in crown-rump length (approximately from the last third of gestation). Tissues were removed from the anterior chamber four to ten weeks after transplantation.

It is interesting that out of thirteen cotransplants, no ovaries were recovered. The number of "takes" in the male and female hosts, if harvested at the end of three weeks, would have been almost equal. However, with time, more of the male host transplants regressed.

After having examined the cotransplants microscopically, it was found that the female hosts had more gonadal tissue "takes" (three out of six) than the male (two out of seven).

Ovary and Testis Cotransplants in Castrate Animals.

Twelve animals, whose gonads had been removed at least two weeks prior to transplantation, received cotransplants of ovary and testis into the right anterior chamber of the eye. Five males and seven females were used. The embryos ranged in crown-rump length from five to seven cm. and remained in the eye from two to five weeks.

Similar to the normal group, only one of twelve cotransplants yielded ovarian tissue (Table 2). There was an overall sixty per cent recovery of testicular tissue with an equal recovery rate from male and female castrates. The per cent age of regressed cotransplants is also similar for both sexes (20% of the males and 29% of the female hosts.)

Table 3

Cotransplants of Ovary and Testis in Pregnant Hosts.

Host	Transplanted Tissue	Weeks in eye	Time of Preg* Weeks after transplantation	Time of Regression Weeks after transplantation
25	R	7	1	4
27	R	7	1	4
35	Male	3	?	
116	R	14	4	?
117	A	14	8	?
119	R	14	4	5
123	R	20	5	11

R = Regressed
A = Absorbed

*The time of pregnancy was counted back from the time of birth of the litter or from the size of the embryos at the time of sacrifice of the host. The length tables of Draper (15) were used to estimate the age of the embryos. It is, therefore, a very rough estimate. Regression was judged by the gross appearance of the transplant as observed in the eye.

Table 4

Cotransplants of Ovary and Testis in Pregnant Hosts.*

	Host	
Embryos	Number of animals	Per Cent
Total	3	100
Male takes	1	33
Female takes	0	0
Absorbed	0	0
Regressed	2	67

*Animals 25, 27 and 35.

None of the cotransplants yielded both ovarian and testicular tissue.

Cotransplants of Ovary and Testes in Pregnant Animals.

Seven animals with cotransplants of ovary and testis were pregnant during the time transplants were in their eyes (Table 3). One animal was sacrificed after three weeks and was found to be pregnant. (Unfortunately, the embryos were not measured and therefore no estimate could be made of the time of pregnancy). The tissue from the eye was found to be testis.

In five other animals that became pregnant from one to five weeks after transplantation, the tissues all regressed, usually about the 4-5 week post-transplantation. However, in one animal the cotransplant had an increase in size about the time of conception. A little red dot could be seen on the transplant. This persisted for three weeks and then disappeared while the rest of the transplant became white and regressed. The transplant regressed completely so that no histological examination could be made. From its gross appearance, it might have been a corpus luteum of pregnancy.

If a comparison is made of the three pregnant animals (Table 4), whose transplants were removed before ten weeks, with the normal (Table 1) and the castrate (Table 2) animals of a similar period, it is seen that the pregnant animals compare to the group of normals as far as the per cent age of testis "takes" is concerned.

Bilateral Gonadal Transplants.

In three spayed females and four castrate males, embryonic gonads of 4.5 to 11.5 cm. in crown-rump length were transplanted to both eyes. After three to four weeks, the transplants were removed. Four of the animals had ovary placed in one eye and testis in the other. In one of

Table 5

Normal hosts with testis transplants

Embryo	Host					
	Male		Female		Total	
	#	%	#	%	#	%
Total	6	100	2	100	8	100
Male takes	2	33	2	100	4	50
Absorbed	3	50	0	0	1	38
Regressed	1	17	0	0	1	12

Table 6

Normal hosts with ovary transplants

Embryo	Host					
	Male		Female		Total	
	#	%	#	%	#	%
Total	6	100	5	100	11	100
Female takes	2	33.3	1	20	3	27
Absorbed	2	33.3	1	20	3	27
Regressed	2	33.3	3	60	5	46

these animals, both ovary and testis were found; in another animal neither tissue was found; in a third animal the ovary regressed and in the last animal, the testis regressed. Two animals had successful testis grafts in both eyes. One animal with ovaries in both eyes did not have any tissue in one eye and only fibrous tissue in the other.

The most interesting result of this experiment is that one out of four testis and ovary transplants was positive for both tissues while none of the cotransplants had both ovary and testis present when the transplant was examined.

Normal Hosts with Testis Transplants.

Eight animals had testis from embryos ten cm. in length transplanted into the anterior chamber of the right eye (Table 5). The tissues were removed in five to seven weeks. In the six males 1/3 of the transplants were recovered and recognizable as testicular tissue while in the two females, all of the transplants were recovered. One of the male host transplants regressed but none of the female host transplants. Although only two animals represented the female host group, the results seen in the normal ovarian-testis cotransplants (Table 1) is the same, with a higher proportion of testis transplants being successful in the normal female host and the ratio of regressions higher in the normal male host.

If the predominance of the male animals in this group (Table 5) was corrected to an equal male and female group, the overall ratio of "takes" would be about 65%.

Normal Hosts with Ovary Transplants.

Of the eleven normal animals transplanted with embryonic ovaries (Table 6), six were males and five females. The embryos ranged from eight to ten cm. in length and remained in the anterior chamber of the

Table 7

Castrate hosts with testis transplants

Embryo	Host					
	Male		Female		Total	
	#	%	#	%	#	%
Total	4	100	4	100	8	100
Male takes	2	50	4	100	6	75
Male absorbed	2	50	0		2	25
Regressed	0	0	0		0	0

Table 8

Castrate hosts with ovary transplants

Embryo	Host					
	Male		Female		Total	
	#	%	#	%	#	%
Total	5	100	5	100	10	100
Female takes	1	20	2	40	3	30
Absorbed	2	40	2	40	4	40
Regressed	2	40	1	20	3	30

right eye from three to seven weeks. The number of "takes" in both sexes as well as the number of regressions were not significantly different. Perhaps with a larger series of animals the slight difference shown may become more evident. However, in general, the embryonic ovarian tissue seems to differ from embryonic testis tissue in transplantability by a lower per centage of "takes" (27% as compared to 65%) and by an equal ratio of "takes" in both sexes as opposed to the higher number of "takes" the male transplant has in the female host.

Bronski (8) has noted a similar lack of success in the transplantation of embryonic guinea pig ovarian tissue to the anterior chamber of the eye of adult animals. Dameron (12), on the other hand, has reported 50-60% "takes" of guinea pig ovary using 30-42 day old embryos transplanted into the anterior chamber of normal adult hosts. He also reports up to 90% "takes" in castrate adult hosts.

Castrate Hosts with Testis Transplants.

Eight castrate animals (four males and four females) received embryonic testis transplants from embryos 6 to 11.5 cm. in size. The transplants were recovered in four to five weeks. The testis tissue grew in all of the females and only in half of the males (Table 7). These results are very similar to the findings in the normal group (Table 5). Of special interest are the female hosts, since all of the testis transplants were recovered in the castrate as well as normal animals.

Castrate Hosts with Ovary Transplants.

Ten castrate animals (five males and five females) had ovaries from embryos 4.5 to 11.5 cm. in C-R length transplanted to the anterior chamber of the right eye (Table 8). The tissues were removed in three to four weeks

and examined histologically. Three out of ten transplants were successful, three regressed and four were absorbed. If a comparison is made to the normal group (Table 6) no major differences are noted.

Embryonic Ovary Transplanted to Adult Testes.

Seven adult guinea pigs had whole ovaries from seven cm. guinea pig embryos implanted into the right testicle with a trocar. Three additional adult guinea pigs had whole ovaries from five to seven cm. embryos transplanted into each testicle. One adult animal had split testes from a seven cm. embryo transplanted to both testicles. The host testes were removed at intervals of two weeks to five months. The only tissue recovered from all of these grafts was a small area of inflammation infiltrated with small round cells. This was from a three week old transplant.

Adult ovaries have been successfully transplanted to the testes in mice (28) and in guinea pigs (39,59). Neonatal ovaries were transplanted into rats by Turner (57). However, Willis (61) could not get embryonic transplants to grow in rat testes. Greene (22), using the rabbit, was successful in transplanting embryonic tissue grown in the testes into a second generation of testes transplants. No gonadal tissue was used in these transplants. Greene (25) also had success in the transplanting of embryonic ovaries into the testicles. In none of the transplants reported in the literature was there any abnormality other than calcification or bone formation encountered (54).

Second Generation Transplants into the Anterior Chamber of the Eye.

Greene (22) has reported on the serial transfer of non-endocrine embryonic transplants into at least two generations without any loss of transplantability. An attempt was made to repeat these experiments using

embryonic gonadal tissue. The tissue used was from a castrate female who had a cotransplant that filled half the anterior chamber of the eye at the end of three weeks (Figure 10). The tissue was removed under sterile conditions and split into four pieces. These pieces were transplanted to the anterior chamber of the eye of two female and one male gonadectomized animals. The tissue was absorbed in less than two weeks in all three cases. There were no signs of infection present. Histological examination of the tissue transplanted revealed only seminiferous tubules surrounded by a lymphocytic reaction. Few spermatogonia were present and the major portion of the tubules consisted of sertoli cells.

Miscellaneous Ovary-Testis Cotransplants.

Two normal male animals had two day neonatal testis cotransplanted with eight cm. embryonic ovaries into the anterior chamber of the same eye. In addition, two normal males had seven cm. embryonic testis cotransplanted with eight cm. embryonic ovaries into the anterior chamber of the eye. Of the former group, only testicular tissue was recovered from one transplant while the other was absorbed in less than two weeks. In the latter group, the cotransplant was absorbed in less than two weeks in one host and regressed after three weeks in the other.

The Role of Embryo Age in Transplantability.

In a control series of thirty-two animals, eight of the animals had transplants of either ovary or testis from embryos five to eight cm. in length and twenty-four had transplants from embryos 9 to 11.5 cm. in length. By using the arbitrary division of 5-8 and 9-11.5 cm., a comparison can be made of an earlier embryonic stage to a later one. The five to eight cm. embryos are approximately from the early period of the last third of

Table 9

Embryo size and transplantability*

Embryo Size	Host (N&C)† Male A/t	%	Female A/t	%	Transplant Testis A/t	%	Ovary A/t	%
5-8 cm.	1/5	20	0/3	0	0/2	0	1/6	17
9-11.5 cm.	5/11	46	3/13	23	4/12	33	4/12	33

*A = Number absorbed
†T = Total number in category (only animals with ovary or testis in one eye).
3 castrate and 5 normals in the 5-8 cm. group.
11 castrate and 13 normals in the 9-11.5 cm. group.

Table 10

Embryo size and transplantability

Embryo Size	Host (N&C) Male R/t	%	Female R/t	%	Transplant Testis R/t	%	Ovary R/t	%
5-8 cm.	2/5	40	1/3	33	0/2	0	3/6	50
9-11.5 cm.	4/11	36	3/13	23	1/12	8	6/12	50

R = Number regressed
T = Total as in Table 9.

Table 11

Embryo Size and transplantability

Embryo Size	Host (N&C) Male t/T	%	Female t/T	%	Transplant Testis t/T	%	Ovary t/T	%
5-8 cm.	2/5	40	2/3	67	2/2	100	2/6	33
9-11.5 cm.	2/11	18	2/13	54	7/12	58	2/12	17

T = Total number as in Table 7.
t = Number of takes.

gestation and the 9 to 11.5 cm. embryos are from the late period of the last third of gestation (15). The animals were grouped in two different ways and then compared as to the ratios of transplants absorbed,* regressed† and "takes"‡.

In Table 9, the ratio of transplants absorbed in the older embryo group is greater in every category (male and female, testes and ovary).

In Table 10, the ratio of the transplants that regressed is analyzed in two different ways. The ratio regressed is similar in both the early and late embryo groups for all categories.

The number of successful "takes" are compared for the younger and older age embryonic transplants in Table 11. The younger group has a higher proportion of "takes" than the older in all columns.

Thus it would appear that there is an inverse relationship between the success of an embryonic gonadal transplant and the age of the embryo. Although these differences are small, the difference in the age of the embryonic tissue was also small (i.e. early and late last third of pregnancy). Perhaps if newborns were compared to embryos removed in the middle of gestation, a greater difference would be shown.

Duration of Transplants.

Turner (57) had intraocular testicular transplants, in rats, produce

---

*Absorbed is defined as describing a transplant that did not show any increase in size and disappeared from the eye in less than two weeks.

†Regressed is defined as describing a transplant that showed signs of vascularity, increase in size and then was either resorbed completely or showed no gonadal tissue on histological section.

‡"Takes" is defined as transplants removed from the eyes after three or more weeks and have evidence of gonadal tissue on histological examination.

hormones for fifteen months. Successful embryonic gonadal intraocular transplants have also persisted for months in Dameron's (13) experiments.

The longest histologically proven male transplant surviving remained in the anterior chamber for eight weeks. No difference could be demonstrated in the ratio of "takes" to regressions as the transplants were allowed to remain in the eye for increasing periods of time (two to ten weeks). However, in three female hosts, with good growths, cotransplants were allowed to stay in the eye for fourteen weeks. After six, seven and ten weeks, the transplants regressed so that at the end of the fourteen week period, no evidence of the transplants was present.

Microscopic Description of the Transplants.

When the histological sections of the transplants were examined, they were grouped into four main categories, as follows:

1. Necrotic tissue - eosinophilic tissue with poor cellular content and large quantities of hyaline-like substance.

2. Infected tissue - large quantities of polymorphonuclear cells present.

3. Regressed tissue - characterized by a round cell infiltrate with or without fibroblastic proliferation (Figures 16, 17).

4. Gonadal tissue - indicated by the presence of ovum or seminiferous tubules (and in one case, rete-testis). Signs of regressive changes were usually present. The round cell infiltrate was more prominent near the center of the transplant while the fibroblastic proliferation appeared to be spreading from the periphery towards the center.

None of the cotransplants had ovary and testis tissue present in the same eye nor were tumorous growths present.

The ovarian tissue removed from the castrate animals had only

primary follicles (Figures 14, 15). There were well developed granulosa cells in several of these transplants. The normal animals, as a group, had a younger appearing ovary transplant (Figures 12, 13). Many ovum were seen surrounded by a single layer of follicle cells, presenting a picture similar to the ovum found in an embryonic ovary (Figures 3, 4). There were few granulosa cells present in the transplants of normal hosts. At no time were corpora lutea noted in castrates or in normals. This is predictable since corpora lutea normally appear almost two months after birth (7). None of the transplants were allowed to develop for that long a period of time.

The testicular transplants that developed best were in castrate males and females (Figures 6, 7, 8, 9). There was hardly any fibrosis or round cell infiltrate in these transplants. The interstitial cells were more numerous in the castrate host transplants (Figures 6, 7, 8, 9) than in normal host transplants (Figure 5) or in adult testis. However, the seminiferous tubules showed only a single layer of cells (Figure 9). Most of these appeared to be Sertoli cells. Rare spermatogonia were seen. The seminiferous tubules in the normal host transplants were not very different from those in the castrate hosts. Interstitial cells were either absent completely or few in number in the transplants from normal hosts. This was even more marked in both castrates and normal animals when there were round cells or fibroblasts in the close vicinity.

Cystic structures of unknown etiology were noted in some transplants (Figures 14, 18).

SUMMARY OF RESULTS

1. None of the twenty-five cotransplants showed evidence of both ovarian and testicular tissue, whereas one out of four ovary-testis transplants into different eyes of the guinea pig had both gonadal tissues present.

2. Guinea pig ovarian tissue did not grow as well as testicular tissue in the anterior chamber of the eye.

3. In general, there was no marked difference between the number of "takes" of gonadal tissue in castrates and normal hosts.

4. The female appears to be a better host than the male for testis transplants.

5. Gonadal transplants do not survive as well when transplanted together as when transplanted individually.

6. All thirteen embryonic ovaries implanted into adult testes failed to survive.

7. Embryonic gonads from the early part of the last trimester of pregnancy appear to have a slightly greater ratio of successful transplants than gonads from the late part of the third trimester.

8. The longest histologically proven surviving transplant was eight weeks old and of testicular tissue.

9. Microscopic description:

   a) Only primary follicles were seen in recovered ovaries.

   b) Ovarian transplants in the castrate appeared more mature than in normal hosts.

   c) The seminiferous tubules showed only a single layer of cells in most transplants. Sertoli cells predominated. No spermatocytes were seen although rare spermatogonia were present.

d) Several castrate host transplants had Leydig cell hyperplasia.

e) Castrate animals had the better appearing testicular transplants.

f) Regressive changes were characterized by round cell infiltrates and fibroblastic proliferation.

g) No teratomatous or cancerous tissues were produced.

DISCUSSION

The transplantation of embryonic gonads into the anterior chamber of the eye of guinea pigs has been reported by Dameron (13) and Bronski (8). Dameron states that 50-60% of the ovaries or testes can be successfully transplanted into normal adults whereas up to 90% "takes" can be obtained in castrate animals. These contrast with the 66% successful testes grafts in normals, 75% in castrates and a 30% success with ovary grafts in normals and castrates presented in this paper. There are even greater differences if the reports of other species are reviewed. Goodman (17) had 85-100% "takes" of ovaries in adult spayed rats, 90% in normal males and 89-99% in normal females. Dunham and Watts (16), using newborn and adult rat ovaries transplanted to adults, found positive "takes" in over 70% of gonadectomized animals and about 40% in normal animals. Ward (60) used neonatal ovaries transplanted into rats. He had good results in over 90% of the normal females, 80% of the castrate males and 75% of the castrate females.

It is difficult to compare the results of investigators in this field because of the multiplicity of variables involved in transplantation. For the purposes of discussion these variables can be divided into three groups: transplant, host, and technique.

Transplant Factors.

The age of the embryonic tissue is significant. With the exception of Ward (60), who obtained poorer results with young than with older transplants, most investigators found transplants to be more successful when using younger embryonic tissue (41). Whether there is an optimal age for gonadal embryonic transplants has not yet been determined, however, on the

basis of other embryonic transplants, one would expect the best period to be somewhere in the second trimester of pregnancy, after the differentiation of the parenchymal tissues and before the stroma became well differentiated. In addition, it has been observed that rapid growth occurring during this stage favors transplantability (44).

Both the species and the individuality differences are important factors in the transplantability of tissues (41). This is certainly true of adult tissues and probably true, in part, of embryonic tissues. Although Greene (22) has been successful with transplants of embryonic tissues between species (rabbit and guinea pig), Browning (9) has not been able to produce growth of transplants between strains of mice. An antigen-antibody response to foreign protein has been offered as an explanation for the host reaction to the transplanted tissue (11, 21, 27). Greene (23) believes that when embryonic tissue is transplanted, the stroma of the transplant dies and is absorbed, while the parenchyma proliferates as it would in tissue culture. The host tissue then provides the stroma for the transplant as well as some of the vessels. He further states that since the parenchymal tissue of the transplant is less antigenic than the stroma, fewer of the host's defenses would be mobilized and the graft remains viable. The ingrowth of stroma, it seems, is not completely beneficial because the fibroblastic response can overgrow and destroy the transplant (43).

Another factor in the stroma support of transplants is that unless the specialized stromal requirements of a transplant are supplied the transplant will regress. Naturally, the rate of growth of the transplant will also modify its stromal requirement (21).

The specific tissue being transplanted is also of importance. For example, it is much easier for embryonic lung to survive transplantation than endocrine tissues.

Host Factors

The species and individuality differences between the host and the transplant are probably the most important of the host factors. Unless highly inbred strains of animals are used, it is almost impossible to dispense with individuality differences (41, 42). Such difficulties are not insurmountable for embryonic tissues have been transplanted not only to other species but also to other classes of animals (1, 29).

Even in homotransplants there is a species difference. The rate of growth and the number of successful transplants have been noted to be better in the rat than in other experimental animals.

The site of transplantation in the host will also influence the transplant. The brain and the anterior chamber of the eye seem to be the best sites in the body because of the mildness of the host response to the foreign transplant (23, 61). The temperature and the blood supply of the site are also to be considered (56).

No relationship between the time of removal of the transplant and the number of successful "takes" was demonstrated in this paper whereas Loeb (41) feels that the time-lapse will alter the results of transplants.

The sex of the donor in relation to that of the host is important in gonadal transplants. Testes grew better in normal females than in males. No large difference could be ascertained between the ratio of "takes" in gonadectomized and normals in the series presented yet others (12, 26, 44) state that there is a definite stimulus for endocrine tissues to grow in animals deficient in the specific endocrine gland. On the other hand,

Loeb (41) believes that, with the exception of the adrenal cortex, there is no difference in transplantation in animals that have or do not have their endocrine organs.

Finally, the health and nutrition of the host must be maintained for successful transplants.

Technique Factors.

Experimental technique used and the amount of trauma suffered by the transplanted tissue are of obvious importance.

Transplants in Pregnant Hosts

There does not seem to be any agreement in the literature about the influence of pregnancy on the transplant. "In pregnant guinea pigs", writes Loeb, "the reactions against homogenous transplants of thyroid, cartilage, and fat tissue were severe in the majority of cases, even during early pregnancy of the host, but in some animals the reaction was relatively mild." Furthermore, "Freund believed that such tissue (embryonal transplants) grows better in pregnant than in non-pregnant hosts. However, according to Peyton Rous, in the mouse, pregnancy inhibits the growth of transplanted embryonal material in a similar manner to that of tumor transplants" (41). Patino (44), using embryonic adrenal and thyroid tissue, has noted an increase in vascularity and size in previously stationary transplants.

Although the pregnant animals tended to have regression of their transplants in this study, it is difficult to draw any conclusions since the series used was small.

Embryonic Age and Transplantability.

The relationship between embryonic age and transplantability deserves further comment. As early as the 1930's, it was known that the tissue from

very young animals gave better results on transplantation than older animals (42, 61). It was also found that fragments of organs from early uterine life grow more rapidly and to a greater extent than fragments of older organs (22).

Browning (9), using mice, compared transplants of embryonic tissue in the last half of gestation to the transplants of newborn and adult tissues. He found the best growth in thirteen day old embryos; mild growth in sixteen day old embryos; and no growth in newborn or adult homotransplants.

The embryonic transplants persisted indefinitely while the adult tissue was absorbed. In heterotransplants (to mice of another inbred strain) he obtained similar results in respect to growth, but all the transplants regressed.

The results of the comparison of the homotransplantability of younger and older embryos presented in this paper seem to agree with Browning's findings.

It is not known why embryonic tissues manifest such "autonomy" in transplants, while adult tissues do not. Cancers are the only other tissues known to have the property of autonomy. Aside from the accessory factors of cellular vitality and independent metabolism (44), the lack of species specificity on the basis of a low antigenicity is probably of great importance for autonomous growth.

Cotransplantability

An interesting result of the attempt to grow testis and ovary in close association with each other in the same anterior chamber of the eye was the failure of growth of the embryonic ovary. By the laws of

of chance, at least four of the twenty-five animals should have had ovary and testis growing in the same eye,* and about eight should have had some ovary tissue. When ovary and testis were transplanted to separate eyes in the same animal, one out of four animals revealed the presence of both gonadal tissues. It is therefore known that embryonic guinea pig ovary and testis transplants can survive in the same host. Greene (25) has been successful in growing these tissues in close association, in the guinea pig, and Dunham, Watts and Adair (16) performed a similar experiment in the rat. No specific figures are available in these ovary-testis cotransplants.

There are several possible explanations for the lack of growth of the ovaries in the cotransplants. When tissues from two different donors are used, it is possible that we are increasing the intensity of the host response by providing an extra amount of foreign antigen (this is expecially true of mongrel guinea pigs). The host might have responded mildly to the introduction of one foreign tissue but very strongly to two foreign tissues. This explanation, however, is not plausible because it has been shown that there is a flow of antibodies as well as hormones between the blood and the fluid in the anterior chamber (11, 21). Thus, it would be of little difference if the two tissues were in the same eye or in different eyes.

Another reason for gonadal cotransplant incompatibility might

---

*In the control series, the overall recovery of testes was over 60%, while the recovery of ovaries was 30%. Therefore: -ratio testis "takes" x ratio ovary "takes" = ratio ovary-testes "takes -ratio-ovary-testis "takes" x number of cotransplants = number of ovary testis "takes".

3/10 x 6/10 = 18/100

18/100 x 25 = 450/100 = apprx. 4 ovary-testis "takes".

be an adverse effect of the hormones produced by one gonad on the other. Newborn gonadal tissues, transplanted to the eye, have been able to produce hormones (16, 57). However, it is doubtful if they have any effect on each other since embryonic ovaries have been grown in adult testis (25) and adult ovaries have also been known to produce estrogenic substances when transplanted to the testis (38, 39).

The last possibility concerns the growth of the transplants. If the testicular tissue preferentially absorbed the nutrients in the aqueous humor, and overgrew the ovarian tissue, the reason for the difficulty with ovarian growth when in the same anterior chamber with testicular tissue, can be readily perceived. This might also explain why the ovary retains the ability to grow in the anterior chamber of the other eye. Further support to this hypothesis are the facts that the anterior chamber is a better transplantation site for testes than for ovaries (56) and that testicular tissue seems to grow much faster than ovarian tissue when transplanted to the eye (44).

In none of the cotransplants were there any changes suggestive of malignancy. The occasional cystic structure seen was probably due to fallopian tube tissue that was carried along with the ovary into the eye (16). Similar results have been reported in rats (16).

Maturation of the Transplants.

When the transplants were examined microscopically it was noted that the sections from gonadectomized animals had more mature ovaries and interstitial cell hyperplasia of the testes, whereas the seminiferous tubules were composed mainly of supporting cells with a few spermatogonia

and no spermatocytes. Except for the lack of maturation of the seminiferous tubules, these effects would be expected because the castrate and spayed animals produce large quantities of follicle stimulating hormone and luteinizing hormone (49, 51, 52, 57).

Although the temperature of the anterior chamber of the eye approximates that of the scrotum (57), and there probably was an adequate follicle stimulating hormone level in at least the castrate and spayed animals, no spermatogenesis was noted. This may possibly be explained by deleterious local environmental factors.

SUMMARY

1. Embryonic guinea pig gonads were transplanted separately and together into the anterior chamber of the eye of three groups of guinea pigs: normal males and females, castrate and spayed, and pregnant animals. The ratio of successful transplants was compared in each group.

2. A comparison was made between the transplants of embryos of different ages.

3. The microscopic appearance of the transplants was described.

4. The factors involved in transplantation and cotransplantation were reviewed.

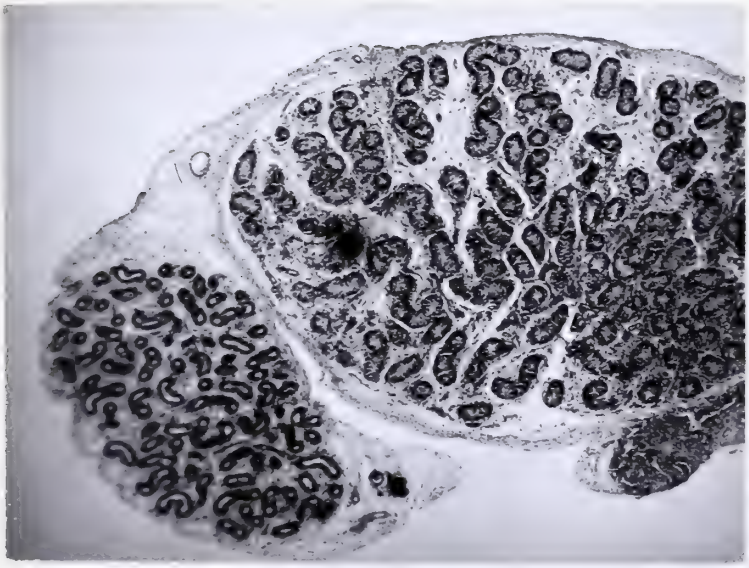


Figure 1. Normal testis from a seven cm. embryonic guinea pig X 27.

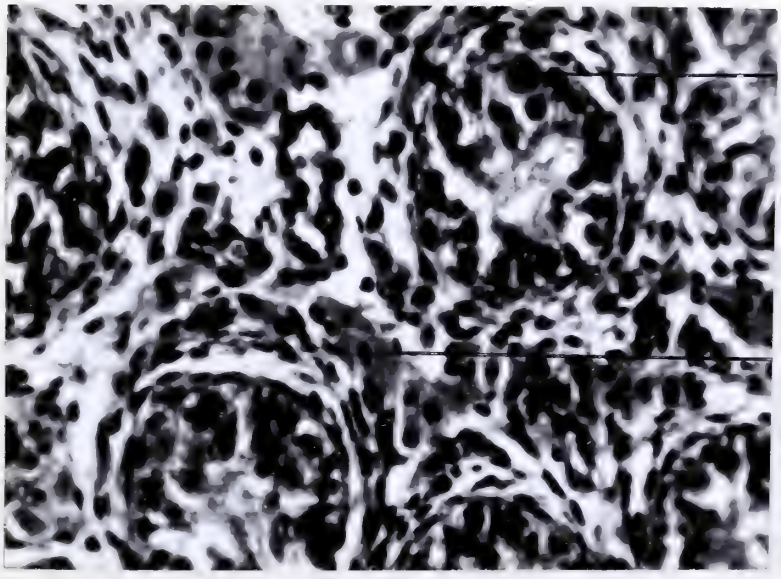


Figure 2. Normal testis from a seven cm. embryonic guinea pig X 405. Note the cellularity of the seminiferous tubules and the clumps of interstitial cells between the tubules. (a) spermatogonia, (b) interstitial cells.

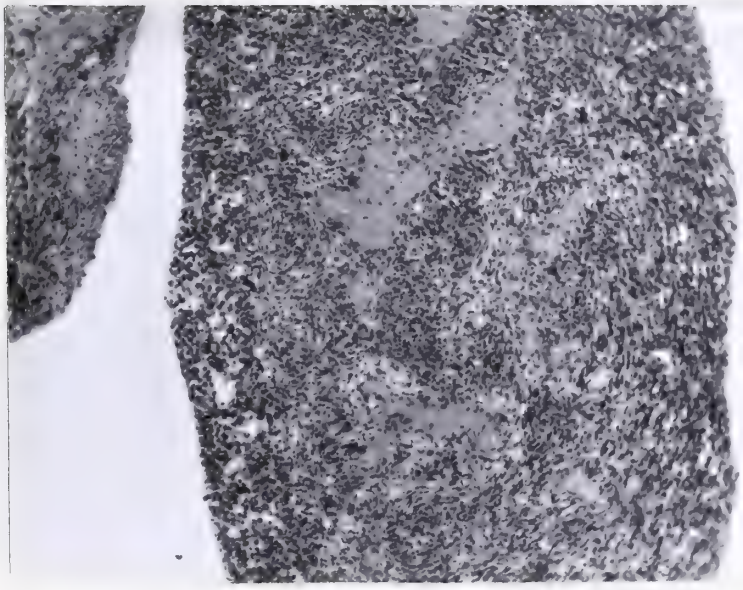


Figure 3. Normal ovary from a seven cm. embryonic guinea pig X 90.

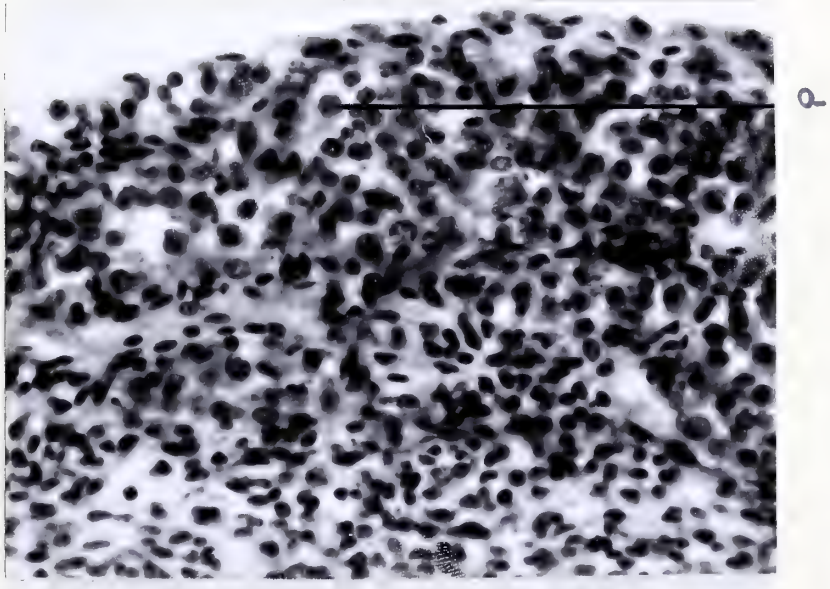


Figure 4. Normal ovary from a seven cm. embryonic guinea pig X 405. (a) early ovum.

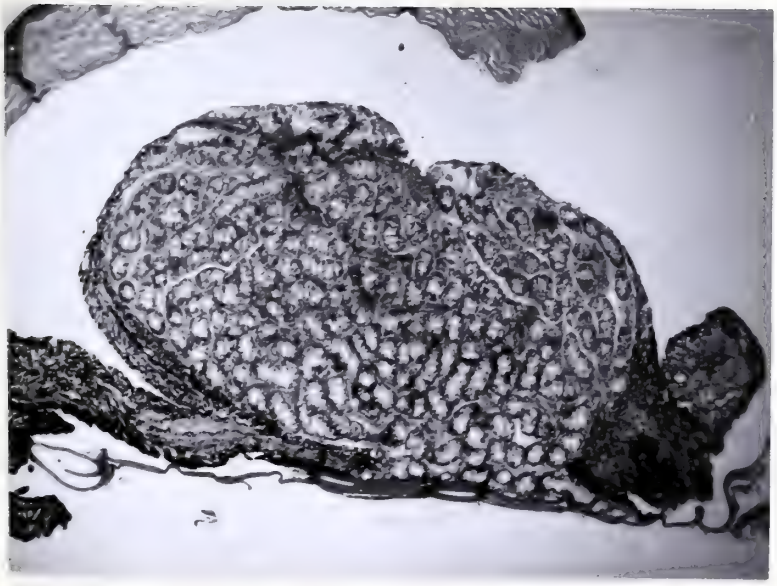


Figure 5. Embryonic testis transplant from a normal female removed after five weeks. X 27.

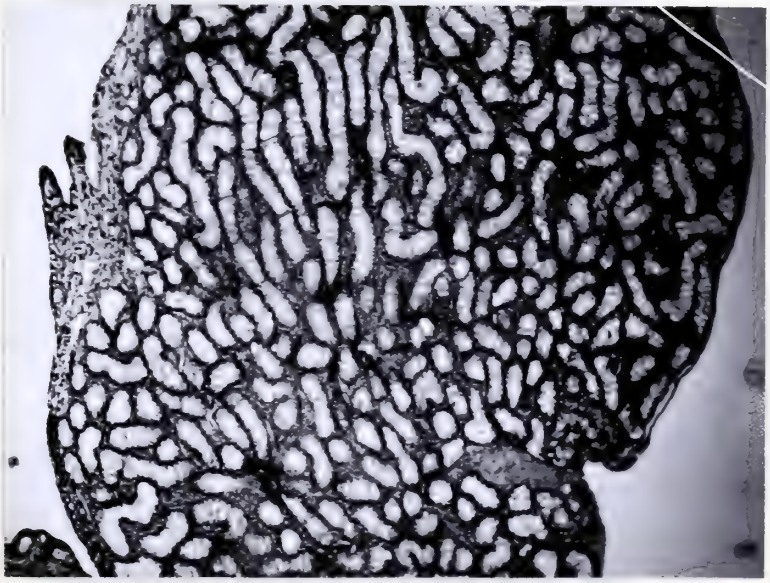


Figure 6. Embryonic testis transplant from a spayed female removed after four weeks. X 27.

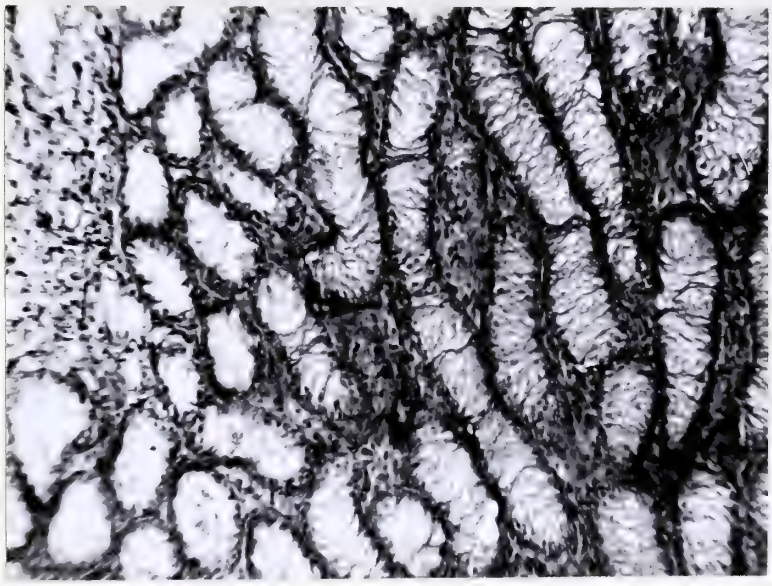


Figure 7. Same transplant as Figure 6 but X 90. Note interstitial cell hyperplasia and the scarcity of cells within the seminiferous tubules.

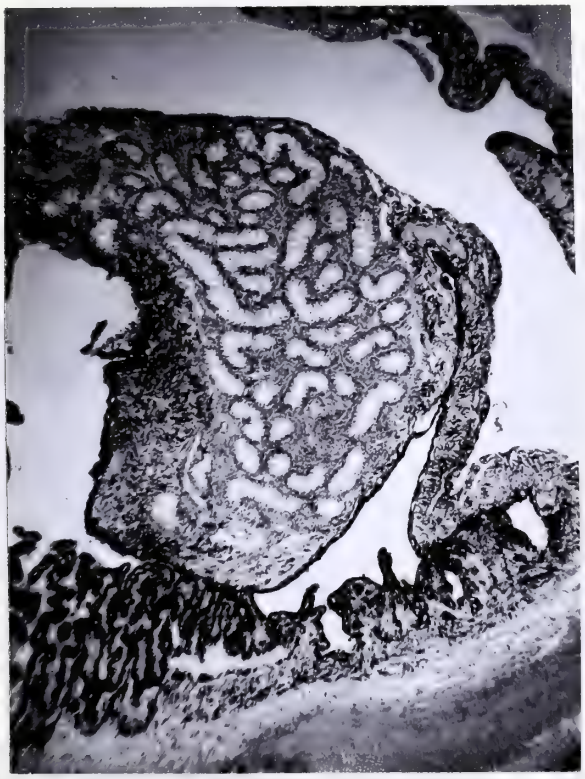


Figure 8. Embryonic testis transplant from a castrated male removed after four weeks X 27.

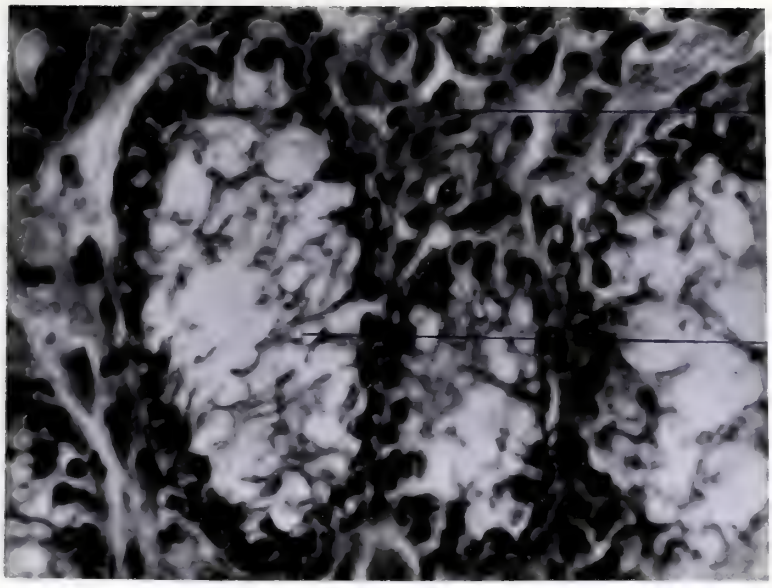


Figure 9. Same as Figure 8 but X 405. Note (a) interstitial cell hyperplasia and (b) the acellularity of the seminiferous tubules.

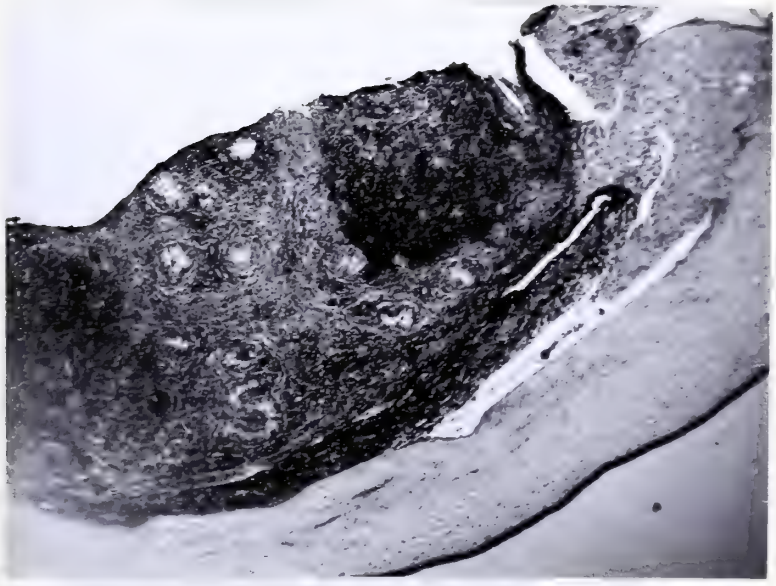


Figure 10. Embryonic ovary-testis cotransplant from a spayed female removed after three weeks. X 27. An attempt was made (without success) to carry this transplant for a second generation.

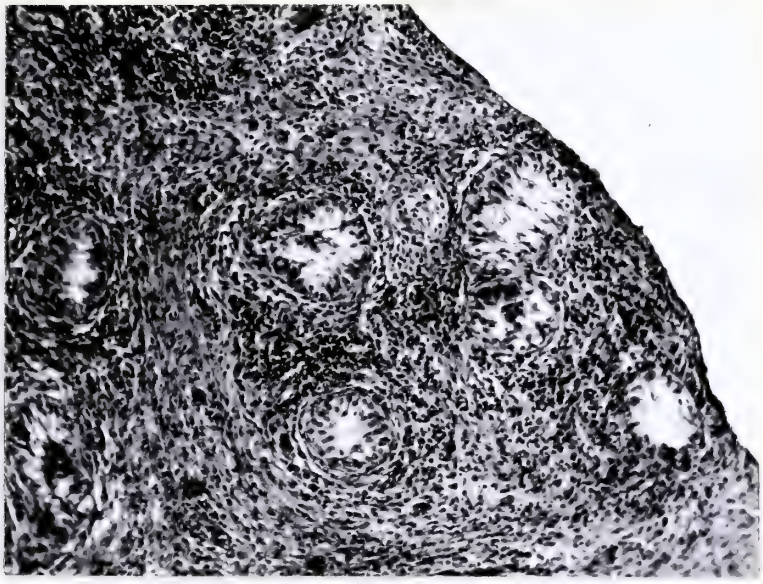


Figure 11. Same as Figure 10 but X 90. Evidence of degeneration is noted by the round cell infiltration.

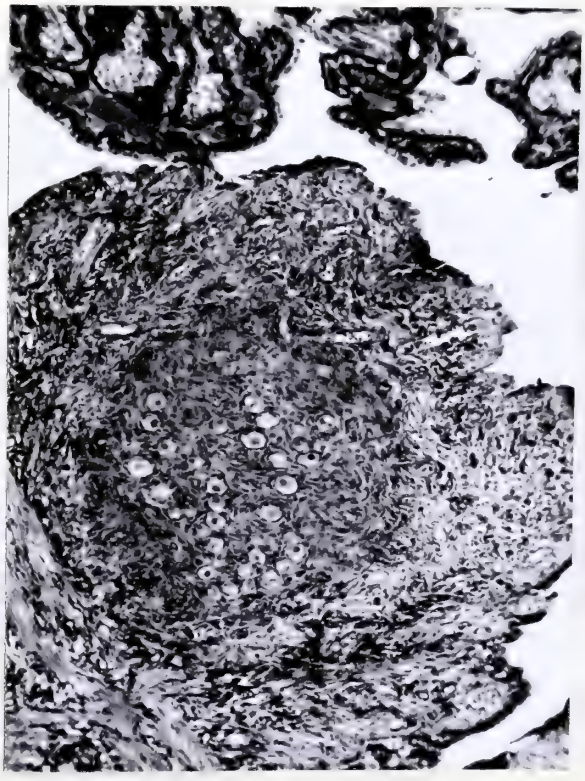


Figure 12. Embryonic ovary transplant from a normal female removed after five weeks. X 27. Many small primary follicles are present. The ova are surrounded by a single layer of follicle cells.

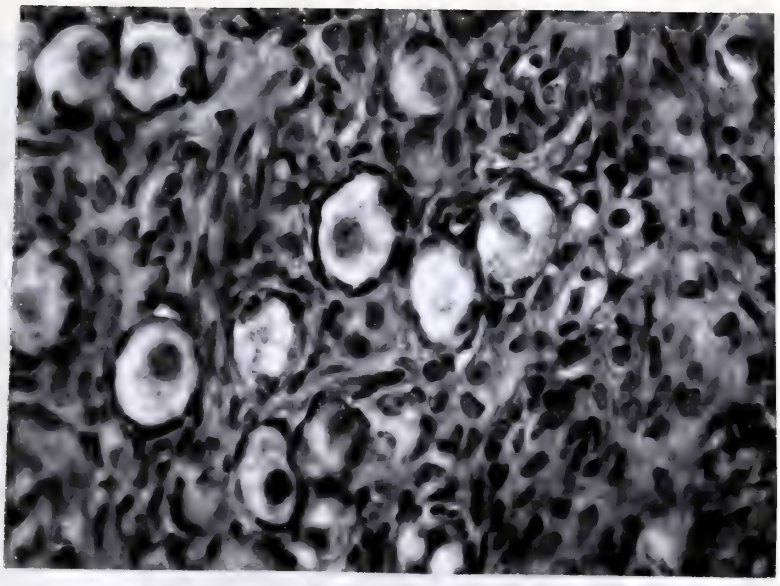


Figure 13. Same as Figure 12 but X 90.

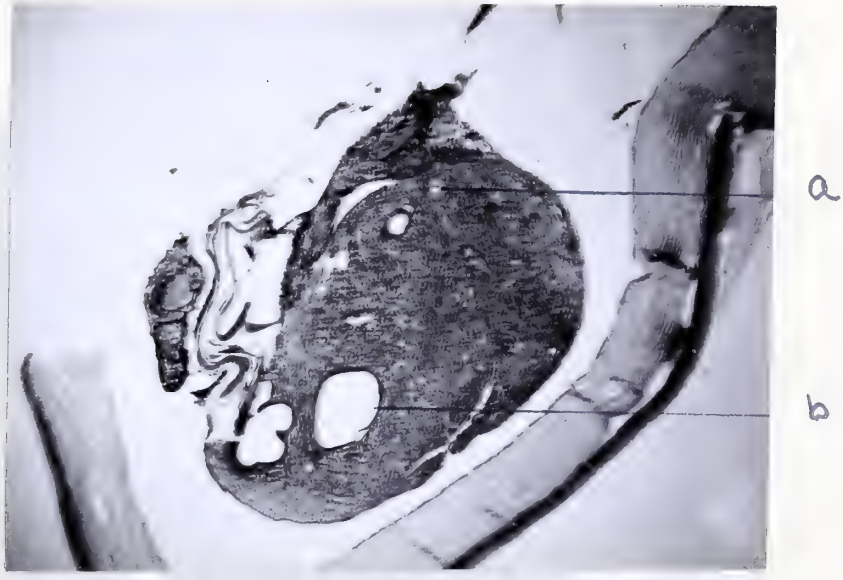


Figure 14. Embryonic ovary transplant from a spayed female removed after four weeks. X 27. (a) ovum (b) cystic structures.

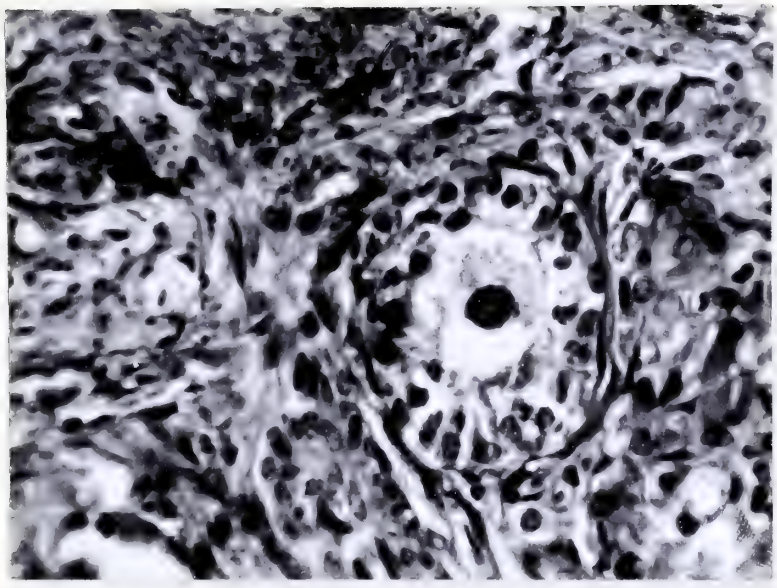


Figure 15. Same as Figure 14 but X 90. Note several layers of follicle cells surrounding the ovum.

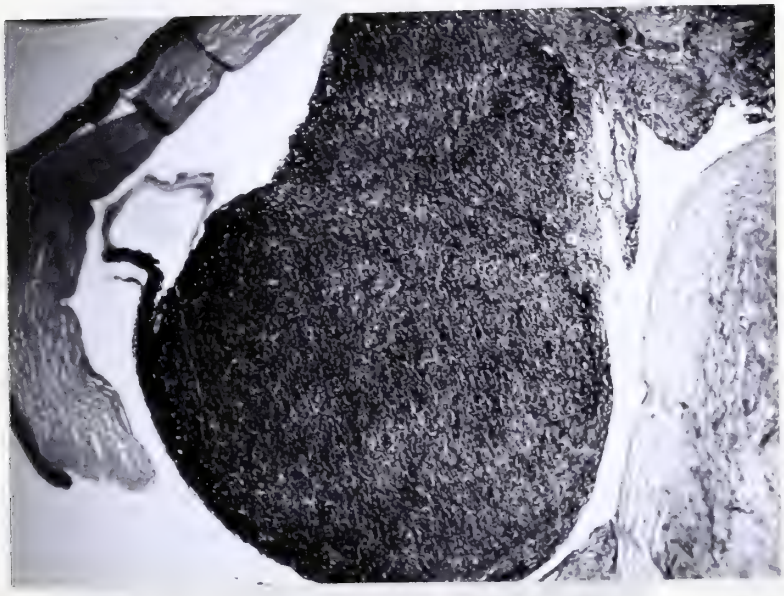


Figure 16. Round cell infiltrate in a cotransplant removed from a normal female after 14 weeks. X 27.

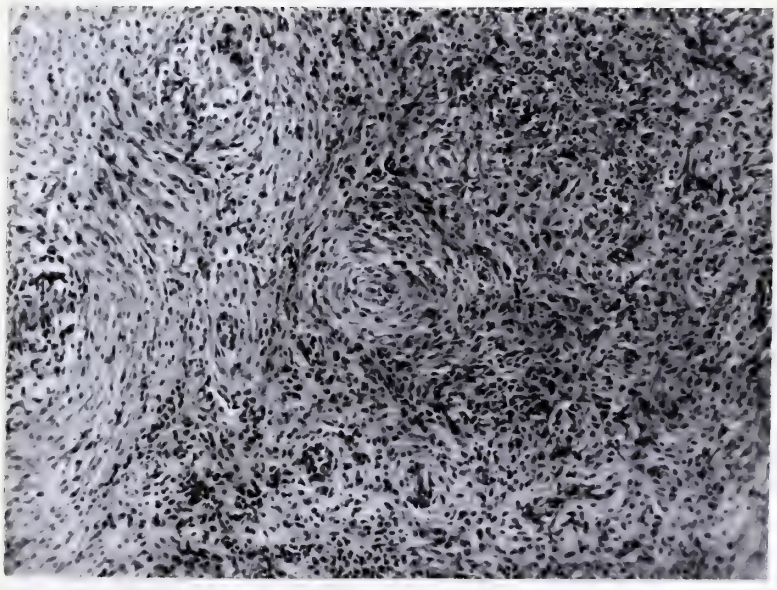


Figure 17. Fibroblastic proliferation in a cotransplant removed from a normal male after four weeks. X 90.

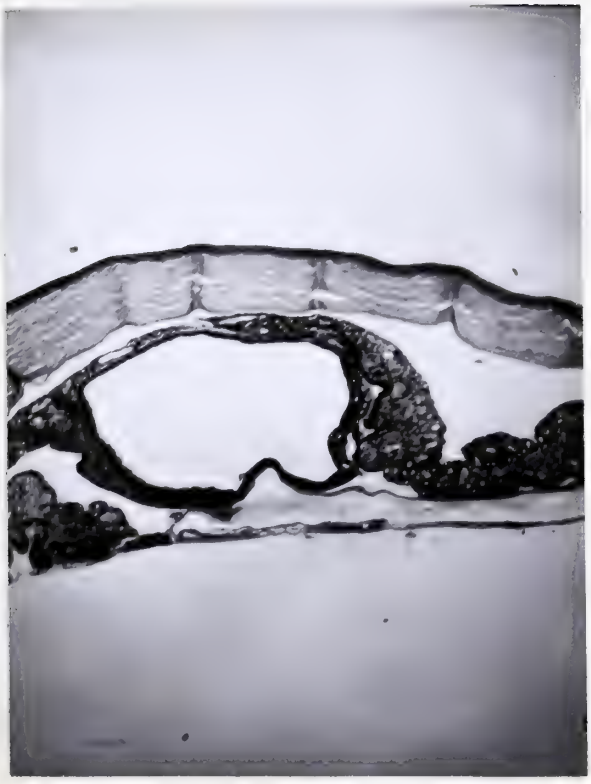


Figure 18. Cystic structure in a cotransplant removed from a castrated male after three weeks. X 27.

References

1. Albrink, W.S. and Greene, H.S.N. The transplantation between zoological classes, Cancer Research 13: 64-68, 1953.

2. Askanazy, M. Centr. Allg. Path. U. Anat. 23: 8, 1912.

3. Atti, F. Allg. Path. U. Path. Anat. 29: 5, 1918.

4. Bonser, G.M. Malignant tumors of the interstitial cells of the testes in Strong A mice tested with triphenylethanol. J. Path and and Bact. 54: 149-154, 1942.

5. ---------- and Dobson, J.M. The effects of prolonged estrogen administration upon male mice of various strains: Development of testicular tumors in the Strong A strain. J. Path and Bact. 51: 9-22, 1940.

6. Bookhout, C.G. The development of the guinea pig ovary from sexual differentiation to maturity. J. of Morph. 77: 233-254, 1945.

7. ---------- The germ cell cycle in the guinea pig. I. The embryonic development of testis. II. The prostate. Ztschr. F. Zellforsch. Bd. 25: 728-763, 1937.

8. Bronski, N. Transplantation of guinea pig embryonic reproduction tissue into the anterior chamber of the eye in adult hosts. J. Exp. Zool. 124: 347-381, 1953.

9. Browning, H.C. Homologous and heterologous growth of transplants of various tissues during the course of development in the mouse. Cancer 2: 646, 1949.

10. Buyse, A. The differentiation of transplanted mammalian gonad primordia. J. Exp. Zool. 70: 1-41, 1935.

11. Cheever, F.S. and Morgan, H.R. Mechanisms of tumor immunity as investigated by means of intraocular inoculation of the Brown-Pearce carcinoma. Cancer Res. 2: 675-679, 1942.

12. Dameron, J.T. The anterior chamber of the eye for investigation purposes. A site for transplantation of fetal endocrine tissues, cancer, and for the study of tissue reaction. Surgery 30: 787-799, 1951.

13. ---------- Homologous transplantation of fetal endocrine tissues to adult non-related hosts. Proc. Soc. Exp. Biol. & Med. 73: 343-345, 1950.

14. Deanesly, R. Androgenic activity of ovarian grafts in castrated male rats. Proc. Royal Soc. London 126: 122-135, 1938.

15. Draper, R.L. The prenatal growth of the guinea pig. Anat. Rec. 18: 369-392, 1920.

16. Dunham, L.J., Watts, R.M., and Adair, F.L. Development of newborn rat ovaries implanted in the anterior chamber of adult rat's eyes: adult rats used: normal, gonadectomized, and gonadectomized treated with gonadotrophin. Arch of Path 32: 910-927, 1941.

17. Gardner, W.U. The effect of ovarian hormones and ovarian grafts on the mammary glands of male mice. Endocrinology 19: 656, 1935.

18. --------- Testicular tumors in mice of several strains receiving triphenylethylene. Cancer Res. 3: 92-99, 1943.

19. Goodman, L. Observations on transplanted immature ovaries in eyes of adult male and female rats. Anat. Rec. 59: 223-251, 1934.

20. Greene, H.S.N. Heterologous transplantation of mammalian tumors I. The transfer of rabbit tumors to alien species. J. Exp. Med. 73: 461-486, 1941.

21. --------- The participation of the anterior chamber of the eye in resistance phenomena related to tumor growth. Cancer Res. 2: 669-674, 1942.

22. --------- Heterologous transplantation of embryonic mammalian tissues. Cancer Res. 3: 809-822, 1943.

23. --------- The use of the mouse eye in transplantation experiments. Cancer Res. 7: 491, 1947.

24. --------- Production of carcinoma and sarcoma in transplanted embryonic tissues. Science 644-645, 1945.

25. --------- Personal Communication.

26. Halstead, W.S. Auto and homiotransplantation of parathyroid in dogs. J. Exp. Med. 11: 175, 1909.

27. Harris, M. The role of humoral antagonism in heteroplastic transplantation in mammals. J. Exp. Zool. 93: 131, 1943.

28. Hill, R.T. Ovaries secrete male hormones; restoration of castrate type of seminal vesical and prostate glands to normal by grafts of ovaries in mice. Endocrinology 21: 495-502, 1937.

29. Hirawa, Y.K. Studies on grafts of embryonic tissue of the rat on the chorio-allantoic membranes of the chick. J. Exp. Zool. 49: 441, 1927.

30. Holyoke, E.A. The differentiation of embryonic gonads transplanted to the adult omentum in the albino rat. Anat. Res. 103: 675-693, 1949.

31. Hooker, C.W., Gardner, W.U. and Pfeiffer, C.A. Testicular tumors in mice receiving estrogens. J.A.M.A. 115: 443-445.

32. Ibsen, H.L. Prenatal growth in guinea pigs with special reference to environmental factors affecting weight at birth. J. Exp. Zool. 51: 51-91, 1928.

33. Kidd, J.G. Distinctive constituents of tumor cells and their possible relations to the phenomena of autonomy, anaplasia and cancer causation. Cold Sp. Harbor Symp. Quant. Biol. 11: 94, 1947.

34. Klee Rawidowicz, E. The production of malignant tumors by transplantation of embryonal tissues. Deutsche Med. Wochenschr 58[2]: 1439-1440, 1932.

35. Lane, E.E. and Markel, J.E. Responses of ovarian intraocular transplants to gonadotropins. Growth 5: 61-67, 1941.

36. Li, M.H. and Gardner, W.U. Tumors in intrasplenic ovarian transplants in castrate mice. Science 105: 13-15, 1947.

37. Lipshutz, A. and Voss, H.E.V. Intrarenal technique for ovary grafting in experimental hermaphoditism. Compte. Rend. Soc. de. Biol. 90: 1139-1141, 1924.

38. --------- and --------- Antagonism of sex glands in experimental hermaphoditism. Compte. Rend. Soc. de. Biol. 91: 232-233, 1924.

39. ---------, Krause, W., and Voss, H.E.V. Experimental hermaphoditism on quantitative lines. Intratesticular ovarian transplants by the method of Sand and Voss. J. of Physiol. 58: 461-465, 1924.

40. Loeb, L. The causes of cancer. New York Academy of Med. Bull 23: 564-580, 1947.

41. --------- The biological basis of individuality, Springfield, Ill., Baltimore, Md., C.C. Thomas pub. 1945.

42. --------- Transplantation and individuality. Physiol. Rev. 10: 547, 1930.

43. --------- Auto and homotransplantation in the guinea pig kidney. J. of Med. Res. 37: 229-246, 1918.

44. Patino, J.F. Studies on the transplantation of embryonic endocrine tissues. Thesis presented to the faculty of the Yale Univ. School of Med. in candidacy for the degree of Doctor of Medicine. 1952.

45. Petrow, N. and Krotkino, N. Der Heutige Stand unserer Ergebnisse der experimentellen Geschwulsterzeugung mittels Embryonalimpfunger. Zeitschr, Krebsforschellung 34: 123-127, 1932.

46. Pfeiffer, C.A. Factors influencing vitality of grafts and production of sex hormones in male rats. Endocrinology 1: 260-267, 1937.

47. --------- Hormones and transplantation. Am. J. Anat. 58: 195-226, 1936.

48. --------- Functional capacities of ovaries of newborn after transplantation into adult ovariotomized rats. Proc. Soc. Exper. Biol. and Med. 31: 479-481, 1934.

49. --------- and Hooker, C.W. Testicular interstitial overgrowth. Cancer Res. 3: 762, 1943.

50. Rous, P. An experimental comparison of transplanted tumor and a transplanted normal tissue capable of growth. J. Exper. Med. 12: 344, 1910.

51. Sand, K. Experiments on the internal secretion of sexual glands, especially on experimental hermaphroditism. J. of Physiol. 53: 257-263, 1919.

52. Selye, H. Textbook of Endocrinology. Acta Endocrinologia. University of Montreal, Montreal, Canada, 1947.

53. Skubisrewski, L. The growth of transplanted embryonal tissue and origin of neoplasms. Compte Rend. Soc. de Biol. 93: 1398-1400, 1925.

54. Smelser, G.K. The response of guinea pig mammary glands to sex hormones and ovarian grafts, and its bearing on the problem of sex hormone antagonism. Physiol. Zool. 6: 396, 1933.

55. Smith, W.E. and Rous, P. The neoplastic potentialities of mouse embryo tissues. II Contributary experiments. Results with skin of C_3H and Webster-Swiss embryos: General considerations. J. Exp. Med. 81: 621-646, 1945.

56. Torrey, T.W. Intraocular grafts of embryonic gonads of the albino rat. Anat. Rec. 96 (4): 256, 1946.

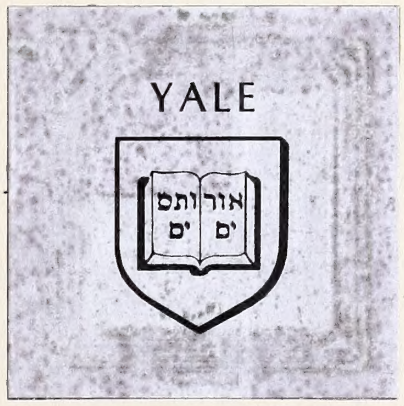
57. Turner, C.D. Intraocular transplantation of prepubertal testes in the rat. Am. J. of Anat. 63: 101-160, 1938.

58. Voss, H.E. Endocrine activities and histological condition of ovarian grafts implanted in testicle. Compt. Rend. Soc. de Biol. 93: 1411-1413, 1925.

59. --------- Histology of experimental ovary-testis. Virchows Archives f. Path. Anat. 261: 425-483, 1926.

60. Ward, J.E., Newton, B.L. and Gardner, H.L. Transplantability of rabbit ovaries in the anterior chamber of the rabbit eye. Proc. of the Soc. for Exper. Biol. and Med. 77: 869-872, 1951.

61. Willis, R.A. Experiments on the intracerebral implantation of embryo tissue in rats. Proc. Royal Soc. of London (B) 117: 400-412, 1935.


YALE
אורותם
ים